1: Biochim Biophys Acta. 1997 Jan 21;1344(2):139-52. Related Articles, Link

Characterization of human apolipoprotein A-I expressed in Escherichia coli.

Bergeron J, Frank PG, Emmanuel F, Latta M, Zhao Y, Sparks DL, Rassart E, Denefle P, Marcel YL.

Lipoproteins and Atherosclerosis Group, University of Ottawa Heart Institute Ontario, Canada.

Human apolipoprotein A-I (apoA-I), with an additional N-terminal extension (Met-Arg-Gly-Ser-(His)6-Met) (His-apoA-I), has been produced in Escherichia coli with a final yield after purification of 10 mg protein/l of culture medium. We have characterized the conformation and structural properties of His-apoA-I in lipid-free form, and in reconstituted lipoproteins containing two apoA-I per particle (Lp2A-I) by both immunochemical and physicochemical techniques. The lipid-free forms of the two proteins present very similar secondary structure and stability, and have also very similar kinetics of association with dimyristoyl phosphatidylcholine. His-apoA-I and native apoA-I can be complexed with 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) to form similar, stable, either discoidal or spherical (sonicated) Lp2A-I particles. Lipid-bound native apoA-I and His-apoA-I showed very similar alpha-helical content (69% and 66%, respectivel in discoidal Lp2A-I and 54% and 51%, respectively in spherical Lp2A-I). Th conformation of His-apoA-I in lipid-free form and in discoidal or spherical Lp2A-I has also been shown to be similar to native apoA-I by immunochemical measurements using 13 monoclonal antibodies recognizing distinct apoA-I epitopes. In the free protein and in reconstituted Lp2A-I, the N-terminal has no effect on the affinity of any of the monoclonal antibodies and minimal effect on immunoreactivity values. Small differences in the exposure of some apoA-I epitopes are evident on discoidal particles, while no difference is apparent in the expression of any epitope of apoA-I on spherical Lp2A-I. The presence of the N-terminal extension also has no effect on the reaction of LCAT with the discoidal Lp2A-I or on the ability of complexes to promote cholesterol efflux from fibroblasts in culture. In conclusion, we show that His-apoA-I expressed in E. coli exhibits similar physicochemical properties to native apoA-I and is also identical to the native protein in its ability to interact with phospholipids and to promote cholesterol esterification and cellular cholesterol efflux.